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<input type="checkbox"/>	L6	(vacuum adj1 dry\$) same liposome\$	74
<input type="checkbox"/>	L5	L1 and 424/450.ccls.	13
<input type="checkbox"/>	L4	(vacuum adj1 dry\$ adj3 without adj3 freez\$)	3
<input type="checkbox"/>	L3	(vacuum adj1 dry\$ adj3 freez\$) same liposome\$	24
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L4: Entry 1 of 3

File: USPT

Sep 26, 1978

DOCUMENT-IDENTIFIER: US 4115928 A

TITLE: Freeze-dry process and product

Detailed Description Text (27):

The underlying physiological mechanism by which the process improves the product is not known. It might be theorized that drawing the vacuum on unfrozen skin opens pores and capillaries and interstitial spaces between the cells. As has been pointed out in the preferred embodiment the vacuum is employed to effect the freezing, but alternative methods of freezing might be successfully employed in lieu of or in augmentation of the vacuum freezing. The subjecting of the skin or other animal tissue to a vacuum may be the crux of the invention in which case it might not be necessary to freeze the tissue while the skin is still subject to vacuum. It might be that the mechanism of the invention is the drawing of water-soluble materials to the surface of the skin during the vacuum drying preceding the sublimation operations, and that the deposit of the water-soluble materials aids reconstitution. In that event, vacuum drying without freezing might yield the product of this invention.

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☐ 1. Document ID: US 4115928 A

Using default format because multiple data bases are involved.

L4: Entry 1 of 3

File: USPT

Sep 26, 1978

US-PAT-NO: 4115928

DOCUMENT-IDENTIFIER: US 4115928 A

TITLE: Freeze-dry process and product

DATE-ISSUED: September 26, 1978

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Reichenbacher; Frank W.	Scottsdale	AZ		

US-CL-CURRENT: 34/287; 34/92, 602/48, 606/229

Full	Title	Citation	Front	Review	Classification	Date	Reference	Sequences	Attachments	Claims	KWIC	Draw D
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☐ 2. Document ID: US 3950560 A

L4: Entry 2 of 3

File: USPT

Apr 13, 1976

US-PAT-NO: 3950560

DOCUMENT-IDENTIFIER: US 3950560 A

TITLE: Method of producing compacted, dehydrated, vegetable products of increased density

DATE-ISSUED: April 13, 1976

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Rahman; Abdul R.	Natick	MA		
Schafer; Glenn R.	Natick	MA		

US-CL-CURRENT: 426/385; 426/454, 426/465, 426/468, 426/615

Full	Title	Citation	Front	Review	Classification	Date	Reference	Sequences	Attachments	Claims	KWIC	Draw D
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☐ 3. Document ID: MX 2002003846 A1, WO 200128522 A2, AU 200074529 A, BR 200015053 A, EP 1221938 A2, HU 200203159 A2, KR 2002063873 A, JP 2003512314 W, CN 1411368 A

L4: Entry 3 of 3

File: DWPI

Oct 1, 2002

DERWENT-ACC-NO: 2001-300273

DERWENT-WEEK: 200370

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TITLE: Producing liposome preparation, having excellent action and redispersion in aqueous medium, by vacuum drying liposome condensed solution without freezing while bubbling or after bubbling condensed solution

INVENTOR: KASAI, A; KONNO, H ; OHTOMO, K

PRIORITY-DATA: 1999JP-0295834 (October 18, 1999)

## PATENT-FAMILY:

PUB-NO	PUB-DATE	LANGUAGE	PAGES	MAIN-IPC
<u>MX 2002003846 A1</u>	October 1, 2002		000	A61K031/436
<u>WO 200128522 A2</u>	April 26, 2001	E	039	A61K009/127
<u>AU 200074529 A</u>	April 30, 2001		000	A61K009/127
<u>BR 200015053 A</u>	July 2, 2002		000	A61K009/127
<u>EP 1221938 A2</u>	July 17, 2002	E	000	A61K009/127
<u>HU 200203159 A2</u>	December 28, 2002		000	A61K009/127
<u>KR 2002063873 A</u>	August 5, 2002		000	A61K009/127
<u>JP 2003512314 W</u>	April 2, 2003		036	A61K009/127
<u>CN 1411368 A</u>	April 16, 2003		000	A61K009/127

INT-CL (IPC): A61 K 9/127; A61 K 31/4353; A61 K 31/436; A61 K 47/24

Full	Title	Citation	Front	Review	Classification	Date	Reference	Sequences	Attachments	Claims	KWIC	Draw D
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(vacuum adj1 dry\$ adj3 without adj3 freez\$)

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L6: Entry 17 of 74

File: USPT

Oct 8, 2002

DOCUMENT-IDENTIFIER: US 6461586 B1

TITLE: Method of magnetic resonance focused surgical and therapeutic ultrasound

Brief Summary Text (134):

In addition to the simple shaking methods described above, more elaborate, but for that reason less preferred, methods can also be employed, e.g., liquid crystalline shaking gas instillation processes, and vacuum drying gas instillation processes, such as those described in U.S. Ser. No. 076,250, filed Jun. 11, 1993, which is incorporated herein by reference, in its entirety. When such processes are used, the stabilized vesicles which are to be gas filled, may be prepared prior to gas installation using any one of a variety of conventional liposome preparatory techniques which will be apparent to those skilled in the art. These techniques include freeze-thaw, as well as techniques such as sonication, chelate dialysis, homogenization, solvent infusion, microemulsification, spontaneous formation, solvent vaporization, French pressure cell technique, controlled detergent dialysis, and others, each involving preparing the vesicles in various fashions in a solution containing the desired active ingredient so that the therapeutic, cosmetic or other agent is encapsulated in, enmeshed in, or attached the resultant polar-lipid based vesicle. See, e.g., Madden et al., Chemistry and Physics of Lipids, 1990 53, 37-46, the disclosure of which is hereby incorporated herein by reference in its entirety.

First Hit    Fwd Refs



L6: Entry 18 of 74

File: USPT

Sep 3, 2002

DOCUMENT-IDENTIFIER: US 6443898 B1

TITLE: Therapeutic delivery systems

Drawing Description Text (14):

FIG. 13 is a graphical representation of the dB reflectivity of gas-filled liposomes substantially devoid of water in the interior thereof prepared by the vacuum drying gas instillation method, without any drugs encapsulated therein. The data was obtained by scanning with a 7.5 megahertz transducer using an Acoustic Imaging.TM. Model 5200 scanner (Acoustic Imaging, Phoenix, Ariz.), and was generated by using the system test software to measure reflectivity. The system was standardized prior to each experiment with a phantom of known acoustic impedance.

Drawing Description Text (15):

FIG. 14 shows a preferred apparatus for preparing the drug containing vacuum dried gas instilled liposomes, and the drug containing gas-filled liposomes substantially devoid of water in the interior thereof prepared by the vacuum drying gas instillation method.

Detailed Description Text (198):

Certain embodiments of the present invention are directed to therapeutic delivery systems comprising gas-filled liposomes prepared by vacuum drying gas instillation methods and having encapsulated therein a therapeutic (that is, contrast agent or drug containing), such liposomes sometimes being referred to herein as therapeutic containing vacuum dried gas instilled liposomes. The present invention is further directed to therapeutic delivery systems comprising therapeutic-containing gas-filled liposomes substantially devoid of liquid in the interior thereof. This method is performed at the phase transition temperature of the gaseous precursor, wherein the gas is thus provided by a gaseous precursor. The liquid precursor becomes a gas which is instilled into the liposomes at the transition temperature.

Detailed Description Text (199):

This method for preparing the liposomes of the subject invention comprises: (i) placing liposomes encapsulating a therapeutic under negative pressure; (ii) incubating the liposomes under the negative pressure for a time sufficient to remove substantially all water from the liposomes; and (iii) instilling selected gas into the liposomes until ambient pressures are achieved. Methods employing the foregoing steps are referred to herein as the vacuum drying gas instillation methods for preparing drug containing liposomes.

Detailed Description Text (200):

Apparatus is also provided for preparing the liposomes of the invention using the vacuum drying gas instillation methods, said apparatus comprising: (i) a vessel containing liposomes having encapsulated therein a therapeutic; (ii) means for applying negative pressure to the vessel to draw water from the liposomes contained therein; (iii) a conduit connecting the negative pressurizing means to the vessel, the conduit directing the flow of said water; and (iv) means for introducing a gas into the liposomes in the vessel.

Detailed Description Text (201):

The vacuum drying gas instillation method employed to prepare both the subject gas-

filled liposomes prepared by the vacuum drying gas instillation method, and the gas-filled liposomes substantially devoid of water in the interior thereof, contemplates the following process. First, in accordance with the process, the therapeutic containing liposomes are placed under negative pressure (that is, reduced pressure or vacuum conditions). Next, the liposomes are incubated under that negative pressure for a time sufficient to remove substantially all water from the liposomes, thereby resulting in substantially dried liposomes. By removal of substantially all water, and by substantially dried liposomes, as those phrases are used herein, it is meant that the liposomes are at least about 90% devoid of water, preferably at least about 95% devoid of water, most preferably about 100% devoid of water. Although the water is removed, the therapeutic, with its higher molecular weight, remains behind, encapsulated in the liposome. Finally, the liposomes are instilled with selected gas by applying the gas to the liposomes until ambient pressures are achieved, thus resulting in the subject therapeutic containing vacuum dried gas instilled liposomes of the present invention, and the therapeutic containing gas-filled liposomes of the invention substantially devoid of water in the interior thereof. By substantially devoid of water in the interior thereof, as used herein, it is meant liposomes having an interior that is at least about 90% devoid of water, preferably at least about 95% devoid of water, most preferably about 100% devoid of water.

Detailed Description Text (202):

Unexpectedly, the therapeutic containing liposomes prepared in accordance with the methods of the present invention possess a number of surprising yet highly beneficial characteristics. The liposomes of the invention exhibit intense echogenicity on ultrasound, will rupture on application of peak resonant frequency ultrasound (as well as other resonant frequencies of sufficient intensity and duration), are highly stable to pressure, and/or generally possess a long storage life, either when stored dry or suspended in a liquid medium. The gaseous precursor-filled liposomes also have the advantages, for example, of stable particle size, low toxicity and compliant membranes. It is believed that the flexible membranes of the gaseous precursor-filled liposomes may be useful in aiding the accumulation or targeting of these liposomes to tissues such as tumors. Also unexpected is the ability of the liposomes during the vacuum drying gas instillation process to fill with gas and resume their original circular shape, rather than irreversibly collapse into a cup-like shape.

Detailed Description Text (203):

The echogenicity of the liposomes and the ability to rupture the liposomes at the peak resonant frequency using ultrasound permits the controlled delivery of therapeutics to a region of a patient by allowing the monitoring of the liposomes following administration to a patient to determine the transition from liquid precursor to gas, the presence of liposomes in a desired region, and the rupturing of the liposomes using ultrasound to release the therapeutics in the region. Preferably, the liposomes of the invention possess a reflectivity of greater than 2 dB, preferably between about 4 dB and about 20 dB. Within these ranges, the highest reflectivity for the liposomes of the invention is exhibited by the larger liposomes, by higher concentrations of liposomes, and/or when higher ultrasound frequencies are employed. See FIG. 13, which is a graphical representation of the dB reflectivity of gas-filled liposomes substantially devoid of water in the interior thereof prepared by the vacuum drying gas instillation method, without any drugs encapsulated therein. Preferably, the liposomes of the invention have a peak resonant frequency of between about 0.5 MHz and about 10 MHz. Of course, the peak resonant frequency of the gaseous precursor-filled and gas-filled liposomes of the invention will vary depending on the diameter and, to some extent, the elasticity of the liposomes, with the larger and more elastic liposomes having a lower resonant frequency than the smaller and more elastic liposomes.

Detailed Description Text (205):

Also unexpected is the ability of the liposomes during the vacuum drying gas

instillation process to fill with gas and resume their original circular shape, rather than collapse into a cup-shaped structure, as the prior art would cause one to expect. See, e.g., Crowe et al., Archives of Biochemistry and Biophysics, Vol. 242, pp. 240-247 (1985); Crowe et al., Archives of Biochemistry and Biophysics, Vol. 220, pp. 477-484 (1983); Fukuda et al., J. Am. Chem. Soc., Vol. 108, pp. 2321-2327 (1986); Regen et al., J. Am. Chem. Soc., Vol. 102, pp. 6638-6640 (1980).

Detailed Description Text (206):

The therapeutic containing liposomes subjected to the vacuum drying gas instillation method of the invention may be prepared using any one of a variety of conventional liposome preparatory techniques which will be apparent to those skilled in the art. Although any of a number of varying techniques can be employed, preferably the therapeutic containing liposomes are prepared via microemulsification techniques. The liposomes produced by the various conventional procedures can then be employed in the vacuum drying gas instillation method of the present invention, to produce the therapeutic containing liposomes of the present invention.

Detailed Description Text (207):

The materials which may be utilized in preparing liposomes to be employed in the vacuum drying gas instillation method of the present invention include any of the materials or combinations thereof known to those skilled in the art as suitable for liposome construction.

Detailed Description Text (209):

To prepare the therapeutic containing liposomes for vacuum drying gas installation, and by way of general guidance, dipalmitoylphosphatidylcholine liposomes, for example, may be prepared by suspending dipalmitoylphosphatidylcholine lipids in phosphate buffered saline or water containing the therapeutic to be encapsulated, and heating the lipids to about 50.degree. C., a temperature which is slightly above the 41.degree. C. temperature required for transition of the dipalmitoylphosphatidylcholine lipids from a gel state to a liquid crystalline state, to form therapeutic containing liposomes.

Detailed Description Text (213):

The therapeutic containing liposomes thus prepared may then be subjected to the vacuum drying gas instillation process of the present invention, to produce the therapeutic containing vacuum dried gas instilled liposomes, and the therapeutic containing temperature activated gaseous precursor-filled liposomes substantially devoid of water in the interior thereof, of the invention. In accordance with the process of the invention, the therapeutic containing liposomes are placed into a vessel suitable for subjecting to the liposomes to negative pressure (that is, reduced pressure or vacuum conditions). Negative pressure is then applied for a time sufficient to remove substantially all water from the liposomes, thereby resulting in substantially dried liposomes. As those skilled in the art would recognize, once armed with the present disclosure, various negative pressures can be employed, the important parameter being that substantially all of the water has been removed from the liposomes. Generally, a negative pressure of at least about 700 mm Hg and preferably in the range of between about 700 mm Hg and about 760 mm Hg (gauge pressure) applied for about 24 to about 72 hours, is sufficient to remove substantially all of the water from the liposomes. Other suitable pressures and time periods will be apparent to those skilled in the art, in view of the disclosures herein.

Detailed Description Text (216):

The above described method for production of liposomes is referred to hereinafter as the vacuum drying gas instillation process.

Detailed Description Text (218):

If the liposomes are cooled to a temperature below 0.degree. C., it is preferable

that the vacuum drying gas instillation process be carried out with liposomes either initially prepared in the presence of cryoprotectants, or liposomes to which cryoprotectants have been added prior to carrying out the vacuum drying gas instillation process of the invention. Such cryoprotectants, while not mandatorily added, assist in maintaining the integrity of liposome membranes at low temperatures, and also add to the ultimate stability of the membranes. Preferred cryoprotectants are trehalose, glycerol, polyethyleneglycol (especially polyethyleneglycol of molecular weight 400), raffinose, sucrose and sorbitol, with trehalose and propylene glycol being particularly preferred.

Detailed Description Text (219):

It has also been surprisingly discovered that the liposomes of the invention are highly stable to changes in pressure. Because of this characteristic, extrusion of the liposomes through filters of defined pore size following vacuum drying and gas instillation can be carried out, if desired, to create liposomes of relatively homogeneous and defined pore size.

Detailed Description Text (220):

As another aspect of the invention, useful apparatus for preparing the therapeutic containing vacuum dried gas instilled liposomes, and the therapeutic containing gas-filled liposomes substantially devoid of water in the interior thereof, of the invention is also presented. Specifically, there is shown in FIG. 14 a preferred apparatus for vacuum drying liposomes and instilling a gas into the dried liposomes. The apparatus is comprised of a vessel 8 for containing therapeutic containing liposomes 19. If desired, the apparatus may include an ice bath 5 containing dry ice 17 surrounding the vessel 8. The ice bath 5 and dry ice 17 allow the liposomes to be cooled to below 0.degree. C. A vacuum pump 1 is connected to the vessel 8 via a conduit 15 for applying a sustained negative pressure to the vessel. In the preferred embodiment, the pump 1 is capable of applying a negative pressure of at least about 700 mm Hg, and preferably a negative pressure in the range of about 700 mm Hg to about 760 mm Hg (gauge pressure). A manometer 6 is connected to the conduit 15 to allow monitoring of the negative pressure applied to the vessel 8.

Detailed Description Text (227):

Thus the invention contemplates methods for the controlled delivery of therapeutic to a region of a patient comprising: (i) administering to the patient the gas-filled liposomes prepared by vacuum drying gas instillation methods and having encapsulated therein a therapeutic, and/or gas-filled liposomes substantially devoid of water in the interior thereof and having encapsulated therein a therapeutic; (ii) monitoring the liposomes using ultrasound to determine the phase transition of the gaseous precursor from liquid to gas phase and to determine the presence of the liposomes in the region; and (iii) rupturing the liposomes using ultrasound to release the therapeutic in the region.

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L6: Entry 19 of 74

File: USPT

May 21, 2002

DOCUMENT-IDENTIFIER: US 6391452 B1

TITLE: Compositions for nasal drug delivery, methods of making same, and methods of removing residual solvent from pharmaceutical preparations

Detailed Description Text (38):

The pharmaceutical matrix may be any pharmaceutical formulation, including but not limited to, e.g., beads, polymer or nonpolymer processed materials, drug-related raw materials, excipients and final products including biopharmaceuticals, including dry powder microspheres such as gelatin microspheres prepared according to the above process, and oil-based microspheres such as liposomes known in the art. The invention is particularly suitable for use with matrices which tend to physically trap entrained solvent within the matrix, so that procedures such as heat or vacuum drying do not effectively remove the solvent.

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L6: Entry 26 of 74

File: USPT

Apr 24, 2001

DOCUMENT-IDENTIFIER: US 6221337 B1

TITLE: Microbubbles surrounded by a monolayer of negatively charged phospholipids as contrast agents

Brief Summary Text (10):

Ultrasound contrast agents comprising gas-filled liposomes, i.e. liposomes which are substantially devoid of liquid in the interior thereof, and their preparation by a vacuum drying gas instillation method are described in WO-A-9222247. The preparation of such gas-filled liposomes by a gel state shaking gas instillation method is described in WO-A-9428780. A report on gas-filled lipid bilayers composed of dipalmitoylphosphatidyl-choline as ultrasound contrast agents is presented by Unger et al. in Investigative Radiology 29, Supplement 2, S134-S136 (1994).

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L6: Entry 53 of 74

File: USPT

Sep 20, 1994

DOCUMENT-IDENTIFIER: US 5348016 A

**\*\* See image for Certificate of Correction \*\***

TITLE: Apparatus for preparing gas filled liposomes for use as ultrasonic contrast agents

Abstract Text (1):

Contrast agents for ultrasonic imaging comprising gas filled liposomes prepared using vacuum drying gas instillation methods, and gas filled liposomes substantially devoid of liquid in the interior thereof, are described. Methods of and apparatus for preparing such liposomes and methods for employing such liposomes in ultrasonic imaging applications are also disclosed. Also described are diagnostic kits for ultrasonic imaging which include the subject contrast agents.

Brief Summary Text (3):

This invention relates to the field of ultrasonic imaging and, more specifically, to gas filled liposomes prepared using vacuum drying gas instillation methods, and to gas filled liposomes substantially devoid of liquid in the interior thereof. The invention also relates to methods of and apparatus for preparing such liposomes and to methods for employing such liposomes in ultrasonic imaging applications.

Brief Summary Text (14):

Specifically, in one embodiment, the present invention provides ultrasound contrast agents comprising gas filled liposomes prepared by vacuum drying gas instillation methods, such liposomes sometimes being referred to herein as vacuum dried gas instilled liposomes.

Brief Summary Text (16):

In a further embodiment, the subject invention provides methods for preparing the liposomes of the subject invention, said methods comprising: (i) placing liposomes under negative pressure; (ii) incubating the liposomes under the negative pressure for a time sufficient to remove substantially all liquid from the liposomes; and (iii) instilling selected gas into the liposomes until ambient pressures are achieved. Methods employing the foregoing steps are referred to herein as the vacuum drying gas instillation methods.

Brief Summary Text (17):

In a still further embodiment, the invention provides apparatus for preparing the liposomes of the invention using the vacuum drying gas instillation methods, said apparatus comprising: (i) a vessel containing liposomes; (ii) means for applying negative pressure to the vessel to draw liquid from the liposomes contained therein; (iii) a conduit connecting the negative pressurizing means to the vessel, the conduit directing the flow of said liquid; and (iv) means for introducing a gas into the liposomes in the vessel.

Brief Summary Text (20):

Surprisingly, the gas filled liposomes prepared by the vacuum drying gas instillation method, and the gas filled liposomes substantially devoid of liquid in the interior thereof which may be prepared in accordance with the vacuum drying gas instillation method, possess a number of unexpected, but highly beneficial, characteristics. The liposomes of the invention exhibit intense echogenicity on

ultrasound, are highly stable to pressure and/or possess a long storage life, either when stored dry or suspended in a liquid medium. Also surprising is the ability of the liposomes during the vacuum drying gas instillation process to fill with gas and resume their original circular shape, rather than irreversibly collapse into a cup-like shape.

Drawing Description Text (2):

FIG. 1 shows an apparatus according to the present invention for preparing the vacuum dried gas instilled liposomes and the gas filled liposomes substantially devoid of liquid in the interior thereof prepared by the vacuum drying gas instillation method.

Drawing Description Text (3):

FIG. 2 is a graphical representation of the dB reflectivity of gas filled liposomes substantially devoid of liquid in the interior thereof prepared by the vacuum drying gas instillation method. The data was obtained by scanning with a 7.5 megahertz transducer using an Acoustic Imaging.TM. Model 5200 scanner (Acoustic Imaging, Phoenix, Ariz.), and was generated by using the system test software to measure reflectivity. The system was standardized prior to each experiment with a phantom of known acoustic impedance.

Detailed Description Text (2):

The present invention is directed to ultrasound contrast agents comprising gas filled liposomes prepared by vacuum drying gas instillation methods, such liposomes sometimes being referred to herein as vacuum dried gas instilled liposomes. The present invention is further directed to contrast agents comprising gas filled liposomes substantially devoid of liquid in the interior thereof.

Detailed Description Text (3):

The vacuum drying gas instillation method which may be employed to prepare both the gas filled liposomes prepared by the vacuum drying gas instillation method, and the gas filled liposomes substantially devoid of liquid in the interior thereof, contemplates the following process. First, in accordance with the process, the liposomes are placed under negative pressure (that is, reduced pressure or vacuum conditions). Next, the liposomes are incubated under that negative pressure for a time sufficient to remove substantially all liquid from the liposomes, thereby resulting in substantially dried liposomes. By removal of substantially all liquid, and by substantially dried liposomes, as those phrases are used herein, it is meant that the liposomes are at least about 90% devoid of liquid, preferably at least about 95% devoid of liquid, most preferably about 100% devoid of liquid. Finally, the liposomes are instilled with selected gas by applying the gas to the liposomes until ambient pressures are achieved, thus resulting in the subject vacuum dried gas instilled liposomes of the present invention, and the gas filled liposomes of the invention substantially devoid of liquid in the interior thereof. By substantially devoid of liquid in the interior thereof, as used herein, it is meant liposomes having an interior that is at least about 90% devoid of liquid, preferably at least about 95% devoid of liquid, most preferably about 100% devoid of liquid.

Detailed Description Text (5):

Also unexpected is the ability of the liposomes during the vacuum drying gas instillation process to fill with gas and resume their original circular shape, rather than collapse into a cup-shaped structure, as the prior art would cause one to expect. See, e.g., Crowe et al., Archives of Biochemistry and Biophysics, Vol. 242, pp. 240-247 (1985); Crowe et al., Archives of Biochemistry and Biophysics, Vol. 220, pp. 477-484 (1983); Fukuda et al., J. Am. Chem. Soc., Vol. 108, pp. 2321-2327 (1986); Regen et al., J. Am. Chem. Soc., Vol. 102, pp. 6638-6640 (1980).

Detailed Description Text (6):

The liposomes subjected to the vacuum drying gas instillation method of the

invention may be prepared using any one of a variety of conventional liposome preparatory techniques which will be apparent to those skilled in the art. These techniques include freeze-thaw, as well as techniques such as sonication, chelate dialysis, homogenization, solvent infusion, microemulsification, spontaneous formation, solvent vaporization, French pressure cell technique, controlled detergent dialysis, and others. The size of the liposomes can be adjusted, if desired, prior to vacuum drying and gas instillation, by a variety of procedures including extrusion, filtration, sonication, homogenization, employing a laminar stream of a core of liquid introduced into an immiscible sheath of liquid, and similar methods, in order to modulate resultant liposomal biodistribution and clearance. Extrusion under pressure through pores of defined size is, however, the preferred means of adjusting the size of the liposomes. The foregoing techniques, as well as others, are discussed, for example, in U.S. Pat. No. 4,728,578; U.K. Patent Application GB 2193095 A; U.S. Pat. No. 4,728,575; U.S. Pat. No. 4,737,323; International Application PCT/US85/01161; Mayer et al., *Biochimica et Biophysica Acta*, Vol. 858, pp. 161-168 (1986); Hope et al., *Biochimica et Biophysica Acta*, Vol. 812, pp. 55-65 (1985); U.S. Pat. No. 4,533,254; Mayhew et al., *Methods in Enzymology*, Vol. 149, pp. 64-77 (1987); Mayhew et al., *Biochimica et Biophysica Acta*, Vol. 755, pp. 169-74 (1984); Cheng et al., *Investigative Radiology*, Vol. 22, pp. 47-55 (1987); PCT/US89/05040; U.S. Pat. No. 4,162,282; U.S. Pat. No. 4,310,505; U.S. Pat. No. 4,921,706; and Liposomes Technology, Gregoriadis, G., ed., Vol. I, pp. 29-37, 51-67 and 79-108 (CRC Press Inc, Boca Raton, Fla., 1984). The disclosures of each of the foregoing patents, publications and patent applications are incorporated by reference herein, in their entirety. Although any of a number of varying techniques can be employed, preferably the liposomes are prepared via microemulsification techniques. The liposomes produced by the various conventional procedures can then be employed in the vacuum drying gas instillation method of the present invention, to produce the liposomes of the present invention.

#### Detailed Description Text (7):

The materials which may be utilized in preparing liposomes to be employed in the vacuum drying gas instillation method of the present invention include any of the materials or combinations thereof known to those skilled in the art as suitable for liposome construction. The lipids used may be of either natural or synthetic origin. Such materials include, but are not limited to, lipids such as fatty acids, lysolipids, dipalmitoylphosphatidylcholine, phosphatidylcholine, phosphatidic acid, sphingomyelin, cholesterol, cholesterol hemisuccinate, tocopherol hemisuccinate, phosphatidylethanolamine, phosphatidylinositol, lysolipids, sphingomyelin, glycosphingolipids, glucolipids, glycolipids, sulphatides, lipids with ether and ester-linked fatty acids, polymerized lipids, diacetyl phosphate, stearylamine, distearoylphosphatidylcholine, phosphatidylserine, sphingomyelin, cardiolipin, phospholipids with short chain fatty acids of 6-8 carbons in length, synthetic phospholipids with asymmetric acyl chains (e.g., with one acyl chain of 6 carbons and another acyl chain of 12 carbons), 6-(5-cholesten-3.beta.-yloxy)-1-thio-.beta.-D-galactopyranoside, digalactosyldiglyceride, 6-(5-cholesten-3.beta.-yloxy)hexyl-6-amino-6-deoxy-1-thio-.beta.-D-galactopyranoside, 6-(5-cholesten-3.beta.-yloxy)hexyl-6-amino-6-deoxyl-1-thio-.alpha.-D-manno pyranoside, dibehenoylphosphatidylcholine, dimyristoylphosphatidylcholine, dilauroylphosphatidylcholine, and dioleoylphosphatidylcholine, and/or combinations thereof. Other useful lipids or combinations thereof apparent to those skilled in the art which are in keeping with the spirit of the present invention are also encompassed by the present invention. For example, carbohydrates bearing lipids may be employed for in vivo targeting as described in U.S. Pat. No. 4,310,505. Of particular interest for use in the present invention are lipids which are in the gel state (as compared with the liquid crystalline state) at the temperature at which the vacuum drying gas instillation is performed. The phase transition temperatures of various lipids will be readily apparent to those skilled in the art and are described, for example, in Liposome Technology, Gregoriadis, G., ed., Vol. I, pp. 1-18 (CRC Press, Inc. Boca Raton, Fla. 1984), the disclosures of which are incorporated herein by reference in their entirety. In addition, it has been found

that the incorporation of at least a small amount of negatively charged lipid into any liposome membrane, although not required, is beneficial to providing highly stable liposomes. By at least a small amount, it is meant about 1 mole percent of the total lipid. Suitable negatively charged lipids will be readily apparent to those skilled in the art, and include, for example phosphatidylserine and fatty acids. Most preferred for reasons of the combined ultimate ecogenicity and stability following the vacuum drying gas instillation process are liposomes prepared from dipalmitoylphosphatidylcholine.

Detailed Description Text (10):

The liposomes thus prepared may then be subjected to the vacuum drying gas instillation process of the present invention, to produce the vacuum dried gas instilled liposomes, and the gas filled liposomes substantially devoid of liquid in the interior thereof, of the invention. In accordance with the process of the invention, the liposomes are placed into a vessel suitable for subjecting to the liposomes to negative pressure (that is, reduced pressure or vacuum conditions). Negative pressure is then applied for a time sufficient to remove substantially all liquid from the liposomes, thereby resulting in substantially dried liposomes. As those skilled in the art would recognize, once armed with the present disclosure, various negative pressures can be employed, the important parameter being that substantially all of the liquid has been removed from the liposomes. Generally, a negative pressure of at least about 700 mm Hg preferably in the range of between about 700 mm Hg and about 760 mm Hg (gauge pressure), applied for about 24 to about 72 hours, is sufficient to remove substantially all of the liquid from the liposomes. Other suitable pressures and time periods will be apparent to those skilled in the art, in view of the disclosures herein.

Detailed Description Text (12):

The above described method for production of liposomes is referred to hereinafter as the vacuum drying gas instillation process.

Detailed Description Text (14):

If the liposomes are cooled to a temperature below 0.degree. C., it is preferable that the vacuum drying gas instillation process be carried out with liposomes either initially prepared in the presence of cryoprotectants, or liposomes to which cryoprotectants have been added prior to carrying out the vacuum drying gas instillation process of the invention. Such cryoprotectants, while not mandatorily added, assist in maintaining the integrity of liposome membranes at low temperatures, and also add to the ultimate stability of the membranes. Preferred cryoprotectants are trehalose, glycerol, polyethyleneglycol (especially polyethyleneglycol of molecular weight 400), raffinose, sucrose and sorbitol, with trehalose being particularly preferred.

Detailed Description Text (15):

It has also been surprisingly discovered that the liposomes of the invention are highly stable to changes in pressure. Because of this characteristic, extrusion of the liposomes through filters of defined pore size following vacuum drying and gas instillation can be carried out, if desired, to create liposomes of relatively homogeneous and defined pore size.

Detailed Description Text (17):

As another aspect of the invention, useful apparatus for preparing the vacuum dried gas instilled liposomes, and the gas filled liposomes substantially devoid of liquid in the interior thereof, of the invention is also presented. Specifically, there is shown in FIG. 1 a preferred apparatus for vacuum drying liposomes and instilling a gas into the dried liposomes. The apparatus is comprised of a vessel 8 for containing liposomes 19. If desired, the apparatus may include an ice bath 5 containing dry ice 17 surrounding the vessel 8. The ice bath 5 and dry ice 17 allow the liposomes to be cooled to below 0.degree. C. A vacuum pump 1 is connected to the vessel 8 via a conduit 15 for applying a sustained negative pressure to the

vessel. In the preferred embodiment, the pump 1 is capable of applying a negative pressure of at least about 700 mm Hg, and preferably a negative pressure in the range of about 700 mm Hg to about 760 mm Hg (gauge pressure). A manometer 6 is connected to the conduit 15 to allow monitoring of the negative pressure applied to the vessel 8.

Detailed Description Text (25):

Kits useful for ultrasonic imaging in accordance with the present invention comprise gas filled liposomes prepared by a vacuum drying gas instillation methods, and gas filled liposomes substantially devoid of liquid in the interior thereof, in addition to conventional ultrasonic imaging kit components. Such conventional ultrasonic imaging kit components are well known, and include, for example, filters to remove bacterial contaminants or to break up liposomal aggregates prior to administration.

First Hit

L6: Entry 64 of 74

File: EPAB

Dec 23, 1992

DOCUMENT-IDENTIFIER: WO 9222298 A1

TITLE: NOVEL LIPOSOMAL DRUG DELIVERY SYSTEMS

Abstract Text (1):

CHG DATE=19990617 STATUS=O>Drug delivery systems comprising gas filled liposomes prepared using vacuum drying gas instillation methods and having encapsulated therein a drug, and gas filled liposomes substantially devoid of liquid in the interior thereof and having encapsulated therein a drug, are described. Methods of and apparatus for preparing such liposomes and methods for employing such liposomes in drug delivery applications are also disclosed.

First Hit

L6: Entry 68 of 74

File: DWPI

Oct 18, 1995

DERWENT-ACC-NO: 1997-373437

DERWENT-WEEK: 199735

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TITLE: Preparation of liposome(s) for use in medicaments for injection

Basic Abstract Text (1):

Preparation of liposomes for use in injectable medicaments such as carmustine, elemene and ginsenoside comprises either dissolving fat-soluble medicine and liposome matrix in organic solvent and forming a lipid-soluble liquid or dissolving only the liposome matrix in the solvent and making into lipid-soluble liquid which is placed in an ampoule. A water-soluble liquid medicine is simultaneously added and the solvent is removed by vacuum drying and then nitrogen gas is added. A dispersant is then injected into the ampoule which is subsequently sealed and left to stand or oscillated and dispersed into the liposome liquid medicine.

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L6: Entry 68 of 74

File: DWPI

Oct 18, 1995

DERWENT-ACC-NO: 1997-373437

DERWENT-WEEK: 199735

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TITLE: Preparation of liposome(s) for use in medicaments for injection

INVENTOR: MA, S; SU, M ; WANG, H

PATENT-ASSIGNEE: DALIAN INST MEDICINAL SCI (DALIN)

PRIORITY-DATA: 1994CN-0112420 (July 30, 1994)

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## PATENT-FAMILY:

PUB-NO	PUB-DATE	LANGUAGE	PAGES	MAIN-IPC
<input type="checkbox"/> <a href="#">CN 1110134 A</a>	October 18, 1995		000	A61K009/127

## APPLICATION-DATA:

PUB-NO	APPL-DATE	APPL-NO	DESCRIPTOR
CN 1110134A	July 30, 1994	1994CN-0112420	

INT-CL (IPC): [A61 K 9/127](#); [A61 K 31/17](#); [A61 K 35/78](#); [A61 K 47/24](#)

ABSTRACTED-PUB-NO: CN 1110134A

## BASIC-ABSTRACT:

Preparation of liposomes for use in injectable medicaments such as carmustine, elemene and ginsenoside comprises either dissolving fat-soluble medicine and liposome matrix in organic solvent and forming a lipid-soluble liquid or dissolving only the liposome matrix in the solvent and making into lipid-soluble liquid which is placed in an ampoule. A water-soluble liquid medicine is simultaneously added and the solvent is removed by vacuum drying and then nitrogen gas is added. A dispersant is then injected into the ampoule which is subsequently sealed and left to stand or oscillated and dispersed into the liposome liquid medicine.

ABSTRACTED-PUB-NO: CN 1110134A

## EQUIVALENT-ABSTRACTS:

DERWENT-CLASS: B05 B07

CPI-CODES: B04-B01B; B05-B01P; B10-A20; B12-M11F;

NC - 001

OPD - 1993-03-10

ORD - 1994-11-15

PAW - (CHIB-N) CHIBA SEIFUN KK

TI - Dried vesicles used as carrier for drug delivery system - prepd. by  
adding glyco-lipid to phospholipid vesicles and drying under reduced  
pressure without lyophilisation